



# Association of fibulin-3 concentrations with the presence and severity of knee osteoarthritis: A cross-sectional study



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## ABSTRACT

**Background:** Fibulin-3, a member of the extracellular matrix glycoproteins family, negatively regulates chondrocyte differentiation. This study aims to assess the correlation of fibulin-3 concentrations with the presence and severity of knee osteoarthritis.

**Methods:** This cross-sectional study was performed in a population of 209 knee osteoarthritis subjects who received hyaluronic acid treatment and 165 healthy controls. Knee osteoarthritis diagnosis was made according to the criteria of the American College of Rheumatology. Osteoarthritis severity was scored by the Kellgren–Lawrence grading method. Synovial fluid was obtained from the knees of osteoarthritis subjects who received the treatment of hyaluronic acid injection for the first time. Serum and synovial fluid fibulin-3 concentrations were examined by enzyme-linked immunosorbent assay method.

**Results:** Mann–Whitney U-test showed that there were higher serum fibulin-3 concentrations in the case group compared with the controls. Higher serum and synovial fluid fibulin-3 concentrations were found in knee osteoarthritis subjects compared with those with Kellgren–Lawrence grades 2 and 3. Knee osteoarthritis subjects with Kellgren–Lawrence grade 3 had higher serum and synovial fluid fibulin-3 concentrations compared with those with Kellgren–Lawrence grade 2. Serum and synovial fluid fibulin-3 concentrations were significantly correlated with Kellgren–Lawrence grading after Pearson correlation analysis ( $r = 0.532$ ,  $P < 0.001$  and  $r = 0.613$ ,  $P < 0.001$ ). Multinomial logistic regression analysis demonstrated a significant association between serum and synovial fluid fibulin-3 concentrations with Kellgren–Lawrence grades ( $P < 0.001$  and  $P < 0.001$ ).

**Conclusion:** Serum and synovial fluid fibulin-3 concentrations were correlated with the presence and severity of knee osteoarthritis.

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## 1. Introduction

Osteoarthritis (OA), a common degenerative joint disease, has some typical characteristics of articular cartilage degradation, subchondral bone damage, and synovitis [1]. Risk factors such as obesity, trauma, aging, and female gender are considered to play important roles in the development and progression of OA [2]. In addition, recent investigations focus on the potential role of inflammation in OA mechanisms [3].

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The fibulin family forms part of the extracellular matrix (ECM) through interacting with other components of the ECM [4]. Fibulin-3, a member of the fibulin family of secreted extracellular glycoproteins, is encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [5]. Two peptides of fibulin-3, named Fib3-1 and Fib3-2, were demonstrated to be elevated in OA subjects compared with the controls [6]. Therefore, fibulin-3 is hypothesized to be involved in the pathogenesis of OA.

We aim to examine the correlation of fibulin-3 concentrations with the presence and severity of knee OA.

## 2. Materials and methods

### 2.1. Subjects

This study was conducted in a population of 209 knee OA subjects who were all treated with hyaluronic acid injection. Patients received an intra-articular injection of hyaluronic acid (25 mg) once a week for five weeks (HA90:Artz, Seikagaku Kogyo, Tokyo, Japan). Knee OA diagnosis was made according to the criteria of the American College of Rheumatology. Subjects were excluded if they had knee injury or surgery history, osteochondritis dissecans, septic arthritis, osteonecrosis, hyaluronic acid injection, corticosteroid injection, or nonsteroidal anti-inflammatory drugs within the past three months. Two experts blind to the study protocol recorded the disease history of the subjects and confirmed the exclusive criteria. The controls were enrolled from the subjects who had medical check-up in our hospital. The controls had no radiological evidence of OA. This study was approved by the ethics committee of our hospital, and informed consent was obtained from all participants.

OA severity was scored by the Kellgren–Lawrence (KL) grading method. Both knees of all participants were assessed based on the radiographic records. OA was diagnosed as having radiographic knee OA of KL grade  $\geq 2$  in at least one knee. The higher grade of the two knees of OA subjects was used for analysis. Subjects who had KL grades of 0 for both knees were considered to be healthy controls.

### 2.2. Laboratory methods

Venous blood was abstracted at 07:00 h after overnight fasting. Before any treatment of OA, synovial fluid (SF) was obtained from the knee (the knee with the higher grade) of OA subjects who received the treatment of hyaluronic acid injection for the first time. Then the specimen was centrifuged to remove cells and joint debris and stored at  $-80^{\circ}\text{C}$  for further measurement. Serum and SF fibulin-3 (including both isoforms: Fib3-1 and Fib3-2) was measured by enzyme-linked immunosorbent assay (Uscn Life Science Inc., Wuhan, China).

### 2.3. Statistical analysis

The data are presented as means  $\pm$  standard deviation (SD) or median (interquartile range). The variable differences between the cases and controls were determined by chi-square tests, unpaired *t*-test, or Mann–Whitney U-test. Kruskal–Wallis test was performed to compare fibulin-3 differences between knee OA subgroups. Pearson correlation analysis and multinomial logistic regression analysis were used to assess the correlation of fibulin-3 concentrations with KL grades. *P*-values  $<0.05$  were considered to be statistically significant.

## 3. Results

### 3.1. Clinical parameters between the two groups

No age, gender, or body mass index (BMI) differences were found between the case and control groups (Table 1).

**Table 1**

The characteristics of patients with knee osteoarthritis and healthy controls.

Characteristics	Knee OA patients	Healthy controls	<i>P</i>
<i>n</i>	209	165	
Age (years)	60.71 $\pm$ 10.54	59.97 $\pm$ 11.76	0.520
Gender (M/F)	73/136	58/107	0.964
BMI ( $\text{kg}/\text{m}^2$ )	24.37 $\pm$ 3.33	24.11 $\pm$ 2.85	0.430
Fibulin-3 in serum (ng/ml)	32.48 (25.93–39.04)	12.28 (10.06–14.58)	$<0.001$
Fibulin-3 in SF (ng/ml)	9.67 (7.81–11.84)		

BMI, body mass index; F, female; M, male; OA, osteoarthritis; SF, synovial fluid.

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